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(57) Abstract

Oligomers containing deazapurine bases have enhanced ability with respect to forming triplexes as compared with oligomers containing only conventional bases. The deazapurine bases are substituted and unsubstituted forms of 7-deazaxanthine and 7-deazaguanine. The oligomers of the invention are capable of forming triplexes with various target sequences such as HER-2 and HIV sequences by coupling into the major groove of a target DNA duplex at physiological pH. The oligomers of the invention may be incorporated into pharmaceutically acceptable carriers and may be constructed to have any desired sequence, provided the sequence includes at least one deazapurine which enhances the ability of the oligomer to form a triplex with the target sequence. Compositions of the invention can be used as pharmaceuticals to treat various diseases linked to genetic material and can be used for diagnostic purposes in order to detect various disease conditions.

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ENHANCED TRIPLE-HELIX FORMATION DIRECTED BY OLIGONUCLEOTIDES COMPOSED OF 2'-DEOXY-7-DEAZAXANTHOSINE AND 2'-DEOXY-7-DEAZAGUANOSINE AND RELATED ANALOGS

Technical Field

The invention relates generally to novel bases, oligonucleotide-based therapy and diagnosis through triplex binding. More specifically, the invention concerns nucleoside analogs such as 2'-deoxy-7-deazaxanthosine and 2'-deoxy-7-deazaguanosine or related nucleoside analogs and oligomers containing same.

Background Art

Duplex DNA can be specifically bound by oligomers based on a recognizable nucleotide sequence. Two major recognition motifs have been recognized. In an earlier description of a "CT" motif, cytosine residues recognize G-C basepairs while thymine residues recognize A-T basepairs in the duplex. These recognition rules are outlined by Maher III, L.J., et al., Science (1989) 245:725-730; Moser, H.E., et al., Science (1987) 238:645-650. More recently, an additional motif, called herein "GT" recognition, was described by Cooney, M., et al., Science (1988) 241:456-459; Hogan, M.E., et al., EP Publication 375408. In the G-T motif, A-T pairs are recognized by adenyl or thymol residues and G-C pairs by guanyl residues (see Beal, P.A. et al., Science (1990) 251:1360-1363).

In both of these binding motifs, the recognition sequence must align with a sequence as played out on one of the chains of the duplex; thus,

recognition, for example, of an A-T pair by a thymine depends on the location of repeated adenyl residues along one chain of the duplex and thymine series on the other. The recognition does not extend to alternating A-T-A-T sequences; only the adenyl residues on one chain or the other would be recognized. An exception to the foregoing is the recent report by Griffin, L.C., et al., Science (1989) 245:967-971, that limited numbers of guanyl residues can be provided within pyrimidine-rich oligomers and specifically recognize thymine-adenine base pairs; this permits the inclusion of at least a limited number of pyrimidine residues in the homopurine target.

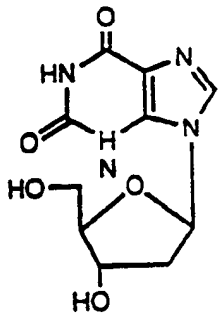
The two motifs exhibit opposite binding orientations with regard to homopurine target chains in the duplex. In the CT motif, the targeting oligonucleotide is oriented parallel to the target sequence; in the GT motif, it is oriented antiparallel (Beal, P.A., et al., Science (1990) 251:1360-1363). Thus, recognition sequences in the CT motif are read with respect to target 5'→3' sequences so that in the 5'→3' direction, synthetic oligonucleotides contain the required sequence of C or T residues with respect to the guanyl or adenyl residues in the target. In the GT motif, on the other hand, the targeted sequence is read 5'→3' in order to design the 3'→5' sequence of the targeting oligonucleotide.

The use of GT motif oligomers for binding to physiological targets such as the human c-myc gene promoter (Postel, E.H. et al., Proc Natl Acad Sci (1991) 88:8227-8231) or the epidermal growth factor receptor gene promoter (Durland, R.H. et al., Biochemistry (1991) 38:9246-9255) have been described. Binding of oligomers to the promoter region was reported to affect the expression of the target gene. This effect was ascribed to interference with promoter activity mediated by the

oligomers. In vitro analyses demonstrated that, under conditions that favored triplex formation, relatively stable triplex structures were formed. However, triplex structures were formed under nonphysiological ion conditions (no K^+ ion present). The present inventors have shown that GT mode oligomers containing only thymine and guanine do not form triplex structures under physiological ion conditions (i.e., 140 mM KCl). The presence of high levels of K^+ ion is postulated by the present inventors to interfere with the formation of triplex structures mediated by guanine-rich oligomers due to the formation of "G-quartets" or similar complex structures (Williamson, J.R. et al., Cell (1989) 59:871-880). As shown below, the oligomers of the present invention that diminish this postulated ion-induced aggregation phenomenon for G-rich oligomers, due to the absence of the nitrogen atom at position 7 of either guanine or xanthine (as a thymine substitute). While not intending to be bound by any theory, the inventors believe this atom mediates chelation of K^+ ion which leads to the postulated aggregation phenomenon.

The present inventors endeavored to substitute the nucleoside analog deoxyxanthosine (shown below as structure A) for thymidine in the G-T motif in order to enhance the association of such oligomers with their complementary duplex DNA.

Structure A



The desired triplex association effect was only achieved under conditions which are more acidic than physiological pH. This shortcoming was rationalized by invoking the ionization of the N-3 proton of the compound of structural formula A at a physiological pH and above.

The present inventors sought to overcome the above-referred to limitation with respect to obtaining association of a given oligomer with DNA duplexes in order to form triplexes at physiological pH. This was done by substituting an analog of the compound A which did not ionize to the same degree under equivalent conditions as did the compound of structure A. (Seela, F., Driller, H., and Liman, U., Liebigs Ann Chemie 1985, 312-320.)

An additional problem relates to the stability of the triplex. Covalent crosslinking to the duplex provides one answer to this problem.

In a recent paper, Praseuth, D., et al., Proc Natl Acad Sci (USA) (1988) 85:1349-1353, described sequence-specific binding of an octathymidylate conjugated to a photoactivatable crosslinking agent to both single-stranded and double-stranded DNA. Use of additional crosslinking agents such as N^4,N^4-ethanocytosine to stabilize triplexes is described in U.S. Serial No. 07/640,654, filed 14 January 1991, which is incorporated herein by reference.

In addition, Vlassov, V.V. et al., Gene (1988) 313-322 and Fedorova, O.S. et al., FEBS (1988) 228:273-276, describe targeting duplex DNA with a 5'-phospho-linked oligonucleotide.

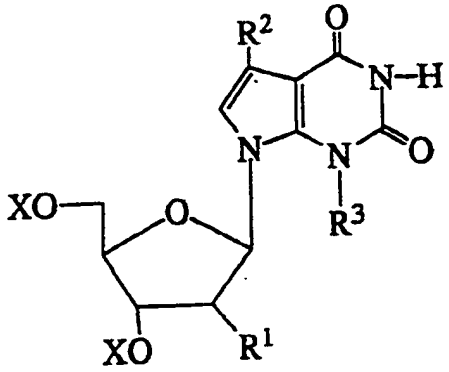
To provide for instances wherein purine residues are concentrated on one chain of the target and then on the opposite chain, oligonucleotides of inverted polarity are provided. Provision of such oligomers is described in U.S. application serial no. 07/559,958,

filed 30 July 1990, and incorporated herein by reference. Briefly, by "inverted polarity" is meant that the oligonucleotide contains tandem sequences which have opposite polarity, i.e., one having polarity 5'→3' followed by another with polarity 3'→5', or vice versa. This implies that these sequences are joined by linkages which can be thought of as effectively a 3'-3' internucleotide junction (however the linkage is accomplished), or effectively a 5'-5' internucleotide junction. Such oligomers have been suggested as by-products of reactions to obtain cyclic oligonucleotides by Capobionco, M.L., et al., Nucleic Acids Res (1990) 18:2661-2669. Compositions of "parallel-stranded DNA" designed to form hairpins secured with AT linkages using either a 3'-3' inversion or a 5'-5' inversion have been synthesized by van de Sande, J.H., et al., Science (1988) 241:551-557. In addition, triple helix formation using an oligomer which contains an effective 3'-3' linkage has been described by Horne, D.A., and Dervan, P.B., J Am Chem Soc (1990) 112:2435-2437.

The various features of oligomer design for triplex formation described in the art may be used to augment the advantages of the improved oligomers described below.

Summary of the Invention

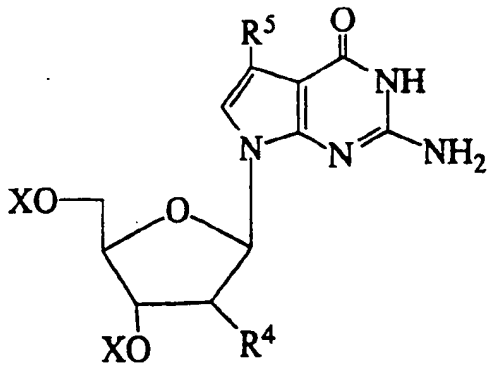
The present invention is directed to deazapurine bases and oligomers containing those bases which oligomers are capable of binding to a variety of desired DNA duplexes and thereby forming triplexes. The oligomers include a nucleoside or nucleotide analog having structural formula I or II. The analog I is defined by the following general structural formula I

I 

wherein R^1 is H, OH, F, Cl, O-allyl, S-allyl, OR or SR, wherein R is alkyl (1-4C); R^2 is H, alkyl(1-4C), CN, Br, Cl, F, $CONR_2$, lower alkenyl(1-4C) or lower alkynyl(1-4C); R^3 is H or a lower alkyl(1-4C); with the proviso that if R^2 and R^3 are both H, R^1 cannot be H or OH, and wherein each X is independently H, $-PO_3^{-2}$ or a group useful in oligomer synthesis.

In preferred embodiments, R^2 is CH_3, R^1 is H or OH and R^3 is H. When one of X is $-PO_3^{-2}$, the compounds of formula I are nucleotides, i.e., deoxyribonucleotides or ribonucleotides. The nucleotides are, of course, formed by the attachment of the phosphate group to the 5' and/or 3' position of the sugar ring--the phosphate group being normally present to link nucleotides to each other and form an oligomer. Further, when oligomers of the invention are synthesized as shown herein, each X is independently a group useful in oligomer synthesis such as DMT, MMT, H-phosphonate, methyl phosphonate, β-cyanoethylphosphoramidite, or methylphosphoramidite.

The analog II is defined by the following general structural formula II

II 

wherein R^4 is H, OH, F, Cl, O-allyl, S-allyl, OR or SR, wherein R is lower alkyl(1-4C); R^5 is H, lower alkyl(1-4C), CN, Br, Cl, F, $CONR_2$, lower alkenyl(1-4C) or lower alkynyl(1-4C); with the proviso that R^5 is not H when R^4 is H or OH;

wherein each X is independently H, $-PO_3^{-2}$ or a group useful in oligomer synthesis.

As above, when one X is $-PO_3^{-2}$, the compounds of formula II are nucleotides, e.g., deoxyribonucleotides and ribonucleotides. Further, when oligomers of the invention are synthesized as shown herein, each X is independently a group useful in synthesis such as DMT, MMT, H-phosphonate, methyl phosphonate, β-cyanoethylphosphoramidite, or methylphosphoramidite.

The analogs of structural formula I or II above are incorporated into oligomers designed for triple-helix formation with a complementary duplex DNA strand. As a result, the oligomers have enhanced ability to form triplexes as compared with oligomers containing only conventional bases. All of the nucleotides in the oligomer may contain the deazapurine bases of the invention. Further, oligomers of the invention may be comprised of residues of both analogs I and II and their analogs.

The oligomers of the invention are capable of forming triplexes with various target sequences such as HER-2 and HIV sequences by coupling into the major groove

of a target DNA duplex at physiological pH. When the triplexes are formed they can prevent or inhibit transcription. The oligomers of the invention may be incorporated into pharmaceutically acceptable carriers and may be constructed to have any desired sequence, provided the sequence includes the deazapurine base residue which enhances the ability of the oligomer to form a triplex with the target sequence. Compositions of the invention can be used as pharmaceuticals to treat various diseases such as cancers (associated with HER-2) and viruses (HIV) and can be used for diagnostic purposes in order to detect the presence of sequences generally known to be associated with neoplastic growth, viruses and a variety of disease conditions.

Thus, the invention is also directed to oligomers which are capable of triple-helix formation containing residues of analogs I and/or II.

Accordingly, in another aspect, the invention is directed to oligomers capable of forming triplexes with various target sequences such as HER-2 and HIV sequences by coupling into the major groove of a target DNA duplex under physiological pH and ion conditions. These oligomers are preferably included in a pharmaceutically acceptable carrier and can have any desired sequence provided the sequence can be modified to include one or more residues of the analogs of structural formula I or II.

In still another aspect, the invention is directed to a method to form a triplex using the oligomers of the invention to target DNA duplexes and to the resulting DNA triplexes. Other aspects of the invention include pharmaceutical and diagnostic compositions which contain the oligomers of the invention, to methods to diagnose and treat diseases

characterized by various target sequences such as HER-2 and HIV duplexes using these compositions.

A primary object of the invention is to provide novel nucleosides and nucleotides and oligomers which include deazapurine bases.

A feature of the invention is that the oligomers of the invention can be comprised of a variety of different sequences and thereby used to target a variety of different target sequences.

Another feature of the invention is that the oligomers may be used by themselves or bound to other compounds such as labels or active pharmaceuticals such as anti-viral or anti-neoplastic agents.

An advantage of the present invention is that the oligomers of the invention are capable of forming triplexes under physiological pH and ion conditions.

Another advantage of the present oligomers is that the novel nucleosides described herein are more hydrophobic than the unmodified parent compounds containing thymine, xanthine or guanine, which hydrophobicity is generally associated with enhanced cellular permeation or uptake when the compounds are administered as therapeutic agents.

These and other objects, advantages and features of the invention will become apparent to those persons skilled in the art upon reading the details of the monomers, oligomers and their synthesis and usage as more fully set forth below, reference being made to the structural formulas and specific examples.

Detailed Description of the Invention

Before the present monomers and oligomers capable of enhanced triplex-helix formation and their synthesis are described, it is to be understood that this invention is not limited to the particular oligomers and

target sequences described as such oligomers and target sequences may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

The invention has been summarized above. Before proceeding with a detailed description of the invention and its methods of synthesis and use, it is believed that it would be useful to provide a definition of some of the terms which will be used throughout this disclosure. Those definitions follow.

As used herein "oligonucleotide" or "oligomer" is generic to polydeoxyribonucleotides (containing 2'-deoxy-D-ribose or modified forms thereof), i.e., DNA, to polyribonucleotides (containing D-ribose or modified forms thereof), i.e., RNA, and to any other type of polynucleotide which is an N-glycoside or C-glycoside of a purine or pyrimidine base, or modified purine or pyrimidine base.

The oligomers of the invention may be formed using conventional phosphodiester-linked nucleotides and synthesized using standard solid phase (or solution phase) oligonucleotide synthesis techniques, which are now commercially available. However, the oligomers of the invention may also contain one or more substitute linkages as is generally understood in the art. These conventional alternative linkages are synthesized as described in the generally available literature.

These alternative linking groups include, but are not limited to embodiments wherein a moiety of the formula P(O)S, $P(O)NR'_2$, P(O)R', $P(O)OR^2$, CO, or $CONR'_2$, wherein R' is H (or a salt) or alkyl (1-20C) and R^2 is alkyl (1-20C) is joined to adjacent nucleotides through -

O- or -S-. Not all such linkages in the same oligomer need to be identical.

Nonphosphorous-based linkages may also be used, such as the formacetal (-O-CH_2-O-), 3'-thioformacetal (-S-CH_2-O_2-), and 3'-amino (-NH-CH_2-O_2-) type linkages described and claimed in copending applications U.S. Serial Nos. 690,786 and 763,130, both assigned to the same assignee as the present application and both incorporated herein by reference.

"Nucleoside" and "nucleotide" include those moieties which contain not only the known purine and pyrimidine bases, but also heterocyclic bases which have been modified. Such modifications include alkylated purines or pyrimidines, acylated purines or pyrimidines, or other heterocycles. Such "analogous purines" and "analogous pyrimidines" are those generally known in the art, many of which are used as chemotherapeutic agents. An exemplary but not exhaustive list includes 5-methyl cytosine, pseudoisocytosine, 8-hydroxy-N-methyladenine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-fluorouracil, 5-bromouracil, 5-carboxymethylamino-methyl-2-thiouracil, 5-carboxymethylaminomethyl uracil, dihydrouracil, inosine, N6-isopentenyl-adenine, 1-methyl-adenine, 1-methylpseudouracil, 1-methylguanine, 1-methyl-inosine, 2,2-dimethylguanine, 2-methyladenine, 2-methyl-guanine, 3-methylcytosine, 5-methylcytosine, N6-methyl-adenine, 7-methylguanine, 5-methylaminomethyl uracil, 5-methoxy aminomethyl-2-thiouracil, beta-D-mannosyl-queosine, 5'-methoxycarbonylmethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid methyl ester, pseudouracil, queosine, 2-thio cytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, N-uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid, pseudouracil, queosine, 2-thiocytosine, 8-oxo-N^6-adenine,

and 2,6-diaminopurine. "Nucleosides" or "nucleotides" also include those which contain modifications in the sugar moiety, for example, wherein one or more of the hydroxyl groups are replaced with halogen, aliphatic groups, or functionalized as ethers, amines, and the like.

The oligomers of the present invention may contain analogous forms of ribose or deoxyribose that are generally known in the art. An exemplary, but not exhaustive, list includes 2'-substituted sugars such as 2'-O-methyl-, 2'-fluoro- or 2'-azido-ribose, alpha-anomeric sugars, epimeric sugars such as arabinose, xyloses or lyxoses, pyranose sugars, sedoheptuloses, acyclic analogs and abasic nucleoside analogs such as methyl riboside.

Furthermore, as the α anomer binds to duplexes in a manner similar to that for the ß anomers, one or more nucleotides may contain this linkage or a domain thereof. (Praseuth, D., et al., Proc Natl Acad Sci (USA) (1988) 85:1349-1353).

Thus, the term "nucleoside" or "nucleotide" will similarly be generic to ribonucleosides or ribonucleotides, deoxyribonucleosides or deoxyribonucleotides, or to any other nucleoside which is an N-glycoside or C-glycoside of a purine or pyrimidine base, or modified purine or pyrimidine base. Thus, the stereochemistry of the sugar carbons may be other than that of D-ribose in one or more residues. Also included are analogs where the ribose or deoxyribose moiety is replaced by an alternate structure such as the 6-member morpholino ring described in U.S. patent number 5,034,506 or where an acyclic structure serves as a scaffold that positions the base analogs described herein in a manner that permits efficient binding to target nucleic acid sequences. The enhanced efficiency of binding by

oligomers containing the base analogs of the present invention is believed to be primarily a function of the base alone. Because of this, elements ordinarily found in oligomers, such as the furanose ring or the phosphodiester linkage may be replaced with any suitable functionally equivalent element.

The oligomers of the present invention also may be of any length, but lengths of 2 to 30 nucleotides are preferred, and those containing about 10-30 nucleotides are more preferred. However, the longer oligonucleotides may also be made, particularly those of greater than 50 nucleotides or greater than 100 nucleotides.

Also included are "derivatives" of oligonucleotides. "Derivatives" of the oligomers include those conventionally recognized in the art. For instance, the oligonucleotides may be covalently linked to various moieties such as intercalators, substances which interact specifically with the minor groove of the DNA double helix and other arbitrarily chosen conjugates, such as labels (radioactive, fluorescent, enzyme, etc.). These additional moieties may be derivatized through any convenient linkage. For example, intercalators, such as acridine can be linked through any available -OH or -SH, e.g., at the terminal 5' position of RNA or DNA, the 2' positions of RNA, or an OH, NH_2, COOH or SH engineered into the 5 position of pyrimidines, e.g., instead of the 5 methyl of cytosine, a derivatized form which contains, for example, $-CH_2CH_2NH_2$, $-CH_2CH_2CH_2OH$ or $-CH_2CH_2CH_2SH$ in the 5 position. A wide variety of substituents can be attached, including those bound through conventional linkages. The indicated -OH moieties in the oligomers may be replaced by phosphonate groups, protected by standard protecting groups, or activated to prepare additional linkages to other nucleotides, or may be bound to the conjugated substituent. The 5' terminal OH may be

phosphorylated; the 2'-OH or OH substituents at the 3' terminus may also be phosphorylated. The hydroxyls may also be derivatized to standard protecting groups.

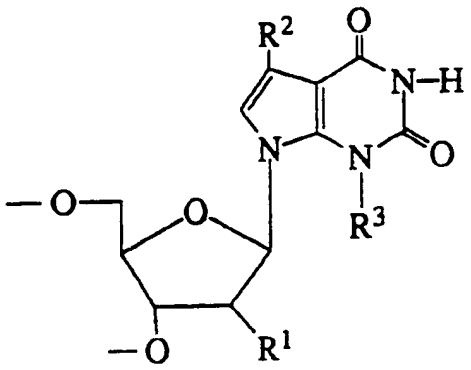
Oligonucleotides or the segments thereof of are conventionally synthesized. Methods for such synthesis are found, for example, in Froehler, B., et al., Nucleic Acids Res (1986) 14:5399-5467; Nucleic Acids Res (1988) 16:4831-4839; Nucleosides and Nucleotides (1987) 6:287-291; Froehler, B., Tet Lett (1986) 27:5575-5578. Variations in the type of internucleotide linkage are achieved by, for example, using the methyl phosphonate precursors rather than the H-phosphonates per se, using thiol derivatives of the nucleoside moieties and generally by methods known in the art.

In addition to employing these very convenient and now most commonly used, solid phase synthesis techniques, oligonucleotides may also be synthesized using solution phase methods such as triester synthesis. These methods are workable, but in general, less efficient for oligonucleotides of any substantial length.

Deazapurine-Containing Monomers and Oligomers

The invention, in one aspect, is directed to the inclusion within an oligomer of a moiety of formula I(a) or II(a), including one or both of these moieties. By including these moieties in an oligomer, it is possible to enhance the ability of the oligomer to bind to various DNA duplexes at physiological pH. More particularly, the invention involves the synthesis, use and incorporation into an oligomer of one or more moieties of formula I(a) or II(a).

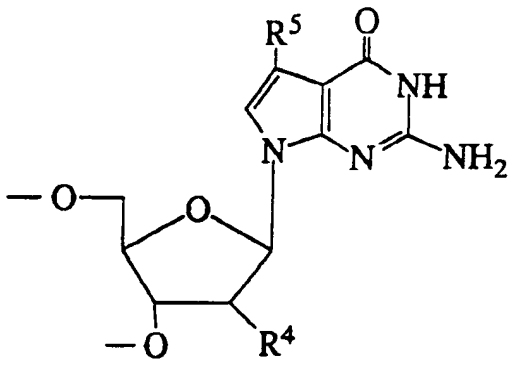
Thus, moieties of formula I(a) are residues of the compounds of formula I as follows:



I(a)

wherein R^1 is H, OH, F, Cl, O-allyl, S-allyl, OR or SR, wherein R is alkyl(1-4C); R^2 is H, alkyl(1-4C), CN, Br, Cl, F, $CONR_2$, lower alkenyl(1-4C) or lower alkynyl(1-4C); and R^3 is H or a lower alkyl(1-4C). In preferred embodiments, R^2 is CH_3, R^1 is H or OH and R^3 is H.

The moiety of formula II(a) is a residue of the compound of formula II:



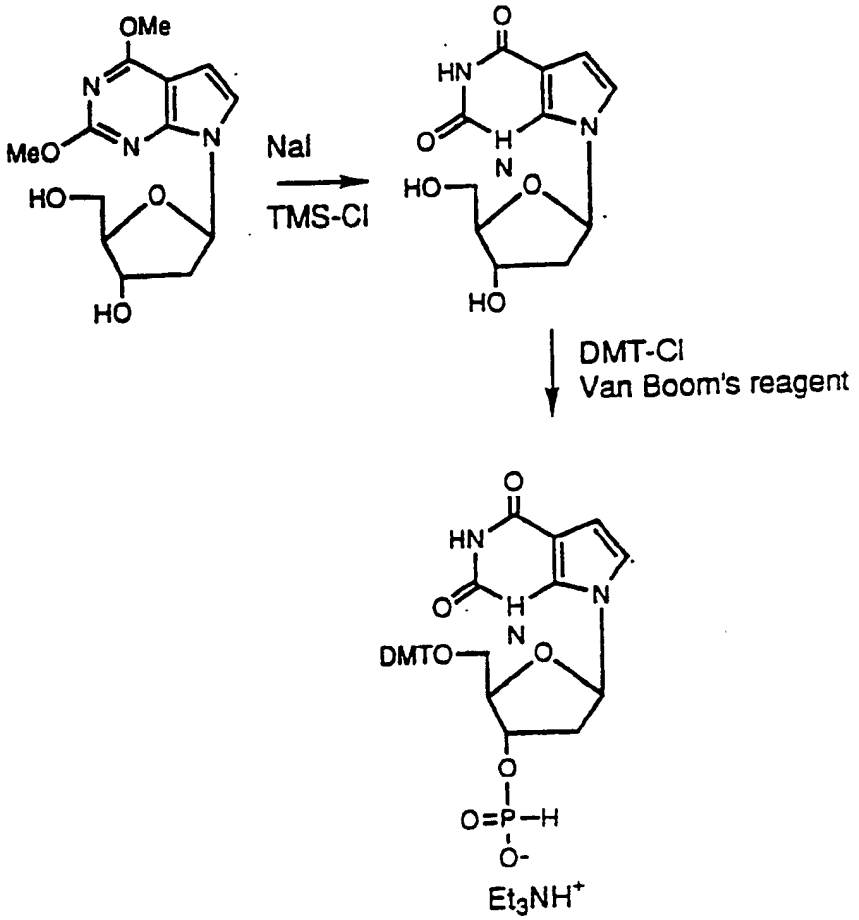
II(a)

wherein R^4 is H, OH, F, Cl, O-allyl, S-allyl, OR or SR, wherein R is lower alkyl(1-4C); and R^5 is H or lower alkyl(1-4C), CN, Br, Cl, F, $CONR_2$, lower alkenyl(1-4C) or

lower alkynyl(1-4C). In preferred embodiments R^4 is H or OH and R^5 is CH_3.

It should be noted that as used in this specification and the appended claims, the singular forms "a", "an", and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a base" includes a plurality of such bases which might be incorporated into an oligomer, reference to "an oligomer" includes a plurality of oligomers and mixtures thereof, and reference to "the method of synthesis" includes any method which might become apparent to those skilled in the art upon reading this disclosure.

Oligomers containing the moiety of formula I(a) can be synthesized by following the general reaction scheme exemplified by 2'-deoxy-7-deazaxanthosine:



Details regarding the synthesis are set forth in Examples 1 and 2.

After synthesizing the intermediate shown above, the intermediate is incorporated into an oligomer designed to bind a target duplex in the GT antiparallel motif. (It has been found that 7-deazaxanthine and 7-deazaguanine containing moieties do not participate in CT mode binding in either direction.)

In a corresponding sequence of reactions, the 2'-deoxy-7-deazaguanosine analog is also protected and phosphorylated and incorporated into oligomers. The analogous nucleosides of the invention, wherein, for example, at least one of R^2 and R^3 or R^5 is other than H, are incorporated in a similar manner.

For synthesis of oligomers useful in forming triplexes with a target DNA duplex, sequences designed to bind the target in the GT mode are provided substituting the monomers of formula I in place of the thymine-containing monomers and monomers of formula II in place of guanine-containing monomers. Thus, nucleotide monomers comprising a base which is a substituted or unsubstituted 7-deazaxanthine will replace nucleotides containing a thymine residue; nucleotides containing a substituted or unsubstituted 7-deazaguanine base will replace nucleotides containing guanine residues.

The oligomers wherein only thymine bases are replaced by substituted or unsubstituted 7-deazaxanthine bases may be referred to as "GX oligomers" which correspond in sequence, except for the indicated base substitution, to the conventionally designed GT oligomer. Similarly, oligomers which correspond in sequence to GT oligomers designed for triplex binding but wherein the G residues are replaced by nucleotides containing the 7-deazaguanine bases, designated G^*, can be referred to as "G^*T oligomers." Of course, both such substitutions could be made, resulting in a G^*X oligomer.

As set forth above, it is not required, in order for the oligomers to fall within the scope of the invention, that each and every G or T be replaced by the analogous nucleotides of the invention containing 7-deazapurine bases.

For example, in designing suitable oligomers for triplex binding to the double-stranded HER-2 sequence,

5' AGGAGAAGGAGGAGG 3',

3' TCCTCTTCCTCCTCC 5',

the oligomer in the conventional system used to effect GT binding would be

5' GGTGGTGGTTGTGGT 3'.

The corresponding GX oligomer of the invention would then be

5' GGXGGXGGXXGXGGX 3'.

The corresponding G*T sequence would then be

5' G*G*TG*G*TG*G*TTG*TG*G*T 3'.

Other oligomers of the invention which would be suitable for binding to this target sequence would include:

G*G*XG*G*XG*G*XXG*XG*G*X;

G*GXGG*XGG*TTG*TG*G*X;

GGXG*G*TGG*XXG*TG*G*X; and

G*G*TGGXG*G*XTGXG*G*T.

Oligomers were synthesized to target the HER2 sequence above; these were of the formulas:

Conventional: 5' GGTGGTGGTTGTGGTY 3',

GX: 5' GGXGGXGGXXGXGGXY 3',

where Y is anthraquinone as further described below. The GX oligomer showed enhanced triplex formation as compared to the native sequences.

Inverted Polarity

As set forth above, it may be useful to synthesize the oligomers containing the deaza bases wherein the oligomers contain regions of inverted polarity in order to accommodate targets wherein in purine-rich sequence occur in complementary regions of the duplex. In their most general form, the inverted polarity oligonucleotides contain at least one segment along their length of the formula:

3'----→5'--C--5'-----3' (1)

or

5'----→3'--C--3'-----5' (2)

where -C- symbolizes any method of coupling the nucleotide sequences of opposite polarity.

In these formulas, the symbol 3'----5' indicates a stretch of oligomer in which the linkages are consistently formed between the 5' hydroxyl of the ribosyl residue of the nucleotide to the left with the 3' hydroxyl of the ribosyl residue of the nucleotide to the right, thus leaving the 5' hydroxyl of the rightmost nucleotide ribosyl residue free for additional conjugation. Analogously, 5'----3' indicates a stretch of oligomer in the opposite orientation wherein the linkages are formed between the 3' hydroxyl of the ribosyl residue of the left nucleotide and the 5' hydroxyl of the ribosyl residue of the nucleotide on the right, thus leaving the 3' hydroxyl of the rightmost nucleotide ribosyl residue free for additional conjugation.

The linkage, symbolized by -C-, may be formed so as to link the 5' hydroxyls of the adjacent ribosyl residues in formula (1) or the 3' hydroxyls of the adjacent ribosyl residues in formula (2), or the "-C-" linkage may conjugate other portions of the adjacent nucleotides so as to link the inverted polarity strands. "-C-" may represent a linker moiety, or simply a covalent bond.

It should be noted that if the linkage between strands of inverted polarity involves a sugar residue, either the 3' or 2' position can be involved in the linkage, and either of these positions may be in either R or S configuration. The choice of configuration will in part determine the geometry of the oligomer in the vicinity of the linkage. Thus, for example, if adjacent 3' positions are used to effect a covalent linkage, less severe deformation of the oligonucleotide chain will generally occur if both 3' hydroxyls involved in the linkage are in the conventional R configuration. If they

are both in the S configuration, this will result in a formable "kink" in the chain.

In addition to the use of standard oligonucleotide synthesis techniques or other couplings to effect the 5'-5' or 3'-3' linkage between ribosyl moieties, alternative approaches to joining the two strands of inverted polarity may be employed. For example, the two appended bases of the opposing termini of the inverted polarity oligonucleotide sequences can be linked directly or through a linker, or the base of one can be linked to the sugar moiety of the other. Any suitable method of effecting the linkage may be employed. The characterizing aspect of the switchback oligonucleotides of the invention is that they comprise tandem regions of inverted polarity, so that a region of 3'→5' polarity is followed by one of 5'→3' polarity, or vice versa, or both.

Depending on the manner of coupling the segments with inverted polarity, this coupling may be effected by insertion of a dimeric nucleotide wherein the appropriate 3' positions of each member of the dimer or the 5' positions of each member of the dimer are activated for inclusion of the dimer in the growing chain, or the conventional synthesis can be continued but using for the condensing nucleotide a nucleotide which is protected/activated in the inverse manner to that which would be employed if the polarity of the chain were to remain the same. This additional nucleotide may also contain a linker moiety which may be included before or after condensation to extend the chain.

The synthesis of oligonucleotides having modified residues and/or inverted polarity may be accomplished utilizing standard solid phase synthesis methods.

In general, there are two commonly used solid phase-based approaches to the synthesis of oligonucleotides containing conventional 3'→5' or 5'→3' linkages, one involving intermediate phosphoramidites and the other involving intermediate phosphonate linkages. In the phosphoramidite based synthesis, a suitably protected nucleotide having a cyanoethylphosphoramidite at the position to be coupled is reacted with the free hydroxyl of a growing nucleotide chain derivatized to a solid support. The reaction yields a cyanoethyl-phosphite, which linkage must be oxidized to the cyanoethylphosphate at each intermediate step, since the reduced form is unstable to acid. The H-phosphonate-based synthesis is conducted by the reaction of a suitably protected nucleoside containing an H-phosphonate moiety at a position to be coupled with a solid phase-derivatized nucleotide chain having a free hydroxyl group, in the presence of a suitable activator to obtain an H-phosphonate diester linkage, which is stable to acid. Thus, the oxidation to the phosphate or thiophosphate can be conducted at any point during the synthesis of the oligonucleotide or after synthesis of the oligonucleotide is complete. The H-phosphonates can also be converted to phosphoramidate derivatives by reaction with a primary or secondary amine in the presence of carbon tetrachloride. To indicate the two approaches generically, the incoming nucleoside is regarded as having an "activated phosphite/phosphate" group.

Variations in the type of internucleotide linkage are achieved by, for example, using the methyl phosphonate precursors rather than the H-phosphonates per se, using thiol derivatives of the nucleoside moieties and generally by methods known in the art. Nonphos-phorous based linkages may also be used, such as the

formacetal, 3'-thioformacetal, and 5'-amino type linkages described and claimed in copending application U.S. Serial No. 690,786 referred to above.

Thus, to obtain an oligonucleotide segment which has a 3'→5' polarity, a nucleotide protected at the 5' position and containing an activated phosphite/phosphate group at the 3' position is reacted with the hydroxyl at the 5' position of a nucleoside coupled to a solid support through its 3'-hydroxyl. The resulting condensed oligomer is deprotected and the reaction repeated with an additional 5'-protected, 3-'phosphite/phosphate activated nucleotide. Conversely, to obtain an oligomeric segment of 5'→3' polarity, a nucleotide protected in the 3' position and containing an activated phosphite/phosphate in the 5' position is reacted with a nucleotide oligomer or nucleoside attached to a solid support through the 5' position, leaving the 3'-hydroxyl available to react. Similarly, after condensation of the incoming nucleotide, the 3' group is deprotected and reacted with an additional 3'-protected, 5'-activated nucleotide. The sequence is continued until the desired number of nucleotides have been added.

In addition to employing these very convenient and now most commonly used, solid phase synthesis techniques, oligonucleotides may also be synthesized using solution phase methods such as triester synthesis. These methods are workable, but in general, less efficient for oligonucleotides of any substantial length.

This oligonucleotide chain elongation will proceed in conformance with a predetermined sequence in a series of condensations, each one of which results in the addition of another nucleotide. Prior to the addition of a nucleoside having an activated phosphite/ phosphate, the protecting group on the solid support-bound nucleotide is removed. Typically, for example, removal

of the commonly-employed dimethoxytrityl (DMT) group is done by treatment with 2.5% v/v dichloroacetic acid/dichloromethane, although 1% w/v trichloroacetic acid/dichloromethane or $ZnBr_2$-saturated nitromethane, are also useful. Other deprotection procedures suitable for other protecting groups will be apparent to those of ordinary skill in the art. The deprotected nucleoside or oligonucleotide bound to solid support is then reacted with the suitably protected nucleotide containing an activated phosphite/ phosphate. After each cycle the carrier bound nucleotide is preferably washed with anhydrous pyridine/ acetonitrile (1:1, v/v), again deprotected, and the condensation reaction is completed in as many cycles as are required to form the desired number of congruent polarity internucleotide bonds which will be converted to phosphoramidates, phosphoro-dithioates, phosphorothioates or phosphodiesters as desired.

In one embodiment, to provide the switchback, the incoming activated, protected nucleoside is provided in the opposite polarity to the support-bound oligomers. Thus, for example, where the support-bound oligomer is 3'→5', the deprotected 5' hydroxyl is reacted with a 3'-protected, 5'-activated monomer, and the synthesis continued with monomers activated at the 5' position and protected at the 3' position.

In another embodiment, to provide a linker in the switchback, a dinucleoside synthon containing the linker element having one end which is activated for condensation (such as a hydrogen phosphonate) to the support-bound oligonucleotide and another end which is a protected hydroxyl group (or protected thio group) is condensed onto the support-bound oligonucleotide. The linked dinucleoside is condensed and deprotected using the same conditions as those used to condense and

deprotect the protected nucleoside hydrogen phosphonate. Subsequent extension of the oligonucleotide chain then uses oligonucleotide residues which are activated and protected in the opposite manner from those used to synthesize the previous portion of the chain.

One approach to this synthesis, using a linker already derivatized to two nucleotide/nucleoside residues which will be included in each portion of the strand is illustrated in Figure 2. The 5'→3' nucleotide portion of the strand is coupled using the 3' DMT-5'-activated phosphate nucleosides, as conventionally, to solid support. The linker is derivatized to two nucleotide residues through their 3' positions; the remaining 5' positions are derivatized by the protecting group DMT in one nucleotide residue and a phosphonate residue in the other. The derivatized linker is coupled to the solid supported strand under standard reagent conditions and then deprotected conventionally. Further standard nucleotide coupling results in extension of the chain in the 3'→5' orientation.

A particularly preferred dimer synthon used to mediate the switchback in an oligomer is the o-xyloso linker. This o-xyloso linker consists of two xylose-nucleosides linked to each other by o-xylene at the 3' position of each xylose sugar. The switchback linker synthon was synthesized using a,a'-dibromoxylene and 5'-DMT nucleoside to give the dimer. The dimer was converted to the H-phosphonate and was used in solid phase synthesis to generate oligomers. Linkers containing the bases guanine, 7-deazaguanine, 7-deazaxanthine, thymine, 5-methylcytosine, 8-hydroxy-N^6-methyladenine, pseudoisocytosine or cytosine are synthesized as homodimers. However, the switchback linker dimers may also be synthesized as mixed

heterodimers that are separated chromatographically or electrophoretically.

Mixed Triplex Binding Motif Oligomers

Another embodiment envisioned for oligomers of the present invention is mixed binding motif oligomers. The target sequence for such oligomers is characterized by a G-rich (≥ ~ 65% guanosine) polypurine region that is adjacent to an A-rich (≥ ~ 65% adenosine) region. The A-rich and G-rich target regions may lie on either the same or on opposite strands, and where opposite strands are to be targeted, a switchback linker may optimally be used in the oligomer. Targets such as these would be efficiently bound by oligomers containing, in one region that binds to the G-rich region of the target, thymidine, guanosine, 2'-deoxy-7-deazaxanthosine, 2'-deoxy-7-deazaguanidine or related nucleotide analogs of the present invention. Such binding would be mediated in the GT binding motif. The other region would consist of nucleotides or nucleotide analogs such as 2'-deoxythymidine, 2'-deoxy-5-methylcytosine, 2'-deoxycytosine, 2'-deoxy-pseudoisocytosine, 2'-deoxy-8-oxo-N^6-methyladenosine or 2'-deoxy-N^4-N^4-ethanocytosine, that bind to A-rich target sequences in the CT binding motif. 2'-deoxy-8-oxo-N^6-methyladenosine is disclosed and claimed in commonly assigned U.S. application serial no. 643,382, which is incorporated herein by reference.

Thus, oligomers containing one region that binds to DNA via CT motif binding may be incorporated adjacent to a region that contains the novel compounds of the present invention. Such oligomers would have continuous polarity but would bind to an A-rich target on one strand that is adjacent to a G-rich target on the other strand. Covalent bonding moieties would

preferentially be incorporated into the CT region of the mixed binding motif oligomer.

Utility and Administration

Oligomers of the invention are capable of significant binding activity to form triplexes or other forms of stable associations. Accordingly, these oligomers are useful in diagnosis and therapy of diseases characterized by the presence of a given genetic sequence such as neoplastic growth (HER-2) and viral infections (HIV).

In these therapeutic applications, the oligomers utilized in a manner appropriate for treatment of the particular disease. For such therapy, the oligomers can be formulated for a variety of modes of administration, including systemic, topical or localized administration. Techniques and formulations generally may be found in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, PA, latest edition. The oligomer itself may be the only active ingredient and is generally combined with a carrier such as a diluent or excipient which may include fillers, extenders, binders, wetting agents, disintegrants, surface-active agents, or lubricants, depending on the nature of the mode of administration and dosage forms. Typical dosage forms include tablets, powders, liquid preparations including suspensions, emulsions and solutions, granules, capsules and suppositories, as well as liquid preparations for injections, including liposome preparations.

For systemic administration, injection is preferred, including intramuscular, intravenous, intraperitoneal, and subcutaneous. For injection, the oligomers of the invention are formulated in liquid solutions, preferably in physiologically compatible buffers such as Hank's solution or Ringer's solution. In

addition, the oligomers may be formulated in solid form and redissolved or suspended immediately prior to use. Lyophilized forms are also included.

Dosages that may be used for systemic administration preferably range from about 0.01 mg/kg to 50 mg/Kg administered once or twice per day. However, different dosing schedules may be utilized depending on (i) the potency of an individual oligomer at inhibiting the activity of its target gene, (ii) the severity or extent of a pathological disease state associated with a given target gene, or (iii) the pharmacokinetic behavior or a given oligomer.

Systemic administration can also be by transmucosal or transdermal means, or the compounds can be administered orally. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, bile salts and fusidic acid derivatives for transmucosal administration. In addition, detergents may be used to facilitate permeation. Transmucosal administration may be through use of nasal sprays, for example, or suppositories. For oral administration, the oligomers are formulated into conventional oral administration forms such as capsules, tablets, and tonics.

For topical administration, the oligomers of the invention are formulated into ointments, salves, gels, or creams, as is generally known in the art.

In addition to use in therapy, the oligomers of the invention may be used as diagnostic reagents to detect the presence or absence of a given target such as HER-2 or HIV sequences to which they specifically bind. Such diagnostic tests are conducted by hybridization through triple helix formation which is then detected by conventional means. For example, the oligomers may be

labeled using any detectable label such as radioactive, fluorescent, or chromogenic labels and the presence of label bound to solid support detected. Alternatively, the presence of a triple helix may be detected by antibodies which specifically recognize these forms. Means for conducting assays using such oligomers as probes are generally known.

The use of oligomers as diagnostic agents by triple helix formation is advantageous since triple helices form under mild conditions and the assays may thus be carried out without subjecting test specimens to harsh conditions. Diagnostic assays based on detection of RNA for identification of bacteria, fungi or protozoa sequences often require isolation of RNA from samples or organisms grown in the laboratory, which is laborious and time consuming; as RNA is extremely sensitive to ubiquitous nucleases.

The oligomer probes may also incorporate additional modifications such as altered internucleotide linkages that render the oligomer especially nuclease stable, and would thus be useful for assays conducted in the presence of cell or tissue extracts which normally contain nuclease activity. Oligonucleotides containing terminal modifications often retain their capacity to bind to complementary sequences without loss of specificity (Uhlmann et al., Chemical Reviews (1990) 90:543-584). The invention probes may also contain linkers that permit specific binding to alternate DNA strands by incorporating a linker that permits such binding (Horne et al., J Am Chem Soc (1990) 112:2435-2437).

Incorporation of base analogs of the present invention into probes that also contain covalent crosslinking agents has the potential to increase sensitivity and reduce background in diagnostic or

detection assays. In addition, the use of crosslinking agents will permit novel assay modifications such as (1) the use of the crosslink to increase probe discrimination, (2) incorporation of a denaturing wash step to reduce background and (3) carrying out hybridization and crosslinking at or near the melting temperature of the hybrid to reduce secondary structure in the target and to increase probe specificity. Modifications of hybridization conditions have been previously described (Gamper et al., Nucleic Acids Res (1986) 14:9943).

In addition to the foregoing uses, the ability of the oligomers to inhibit gene expression can be verified in in vitro systems by measuring the levels of expression in recombinant systems.

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make the compounds and compositions of the invention and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to insure accuracy with respect to numbers used (e.g., amounts, temperatures, etc.), but some experimental errors and deviations should be taken into account. Unless indicated otherwise, parts are parts by weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

Example 1

7-(2'-deoxy-β-D-*erythro*-pentofuranosyl)-7H-pyrrolo[2,3-*d*]pyrimidine-1,3-dione (7-deaza-2'-deoxyxanthosine)

To a solution of 600 mg of 7-(2'-deoxy-β-D-*erythro*-pentofuranosyl)-2,4-dimethoxy-7H-pyrrolo[2,3-*d*]pyrimidine in 20 mL of THF containing 1.2 g of sodium

iodide was added 1.0 mL of trimethylsilyl chloride. The sealed reaction mixture was stirred for 20 hours at room temperature and then quenched with 50 mL of acetonitrile/water 9:1, and the resulting solution was chromatographed on a silica gel column using acetonitrile/water 9:1. The product-containing fraction was evaporated, and the residue was crystallized from water to yield 382 g of the title product.

Example 2

5'-(4,4'-dimethoxytrityl)-7-deaza-2'-deoxyxanthosine-3'-H-phosphonate, triethylamine salt

To a solution of 380 mg of 7-deaza-2'-deoxyxanthosine in 50 mL pyridine was added 950 mg of 4,4'-dimethoxytrityl chloride. The reaction was stirred for 0.5 h, then partitioned between water and ethyl acetate. The organic layer was washed with water and brine, then evaporated. The residue was chromatographed on a silica gel column using methylene chloride/methanol 9:1 to afford 520 mg of a crisp foam. This foam was dissolved in 25 mL of pyridine and the resulting solution was chilled to 0°. The cold solution was treated with 1.5 mL of a 1 M solution of 2-chloro-4H-1,3,2-benzodioxaphosphorin-4-one in methylene chloride. After 15 min, the solution was quenched with 40 mL of ice-cold triethyl ammonium bicarbonate buffer pH 7.5, and the mixture was extracted with methylene chloride. The organic extracts were evaporated and the residue was chromatographed on a silica gel column using acetonitrile/water 9:1 v:v (2% triethylamine) to afford 360 mg of the phosphonate. The protected H-phosphonate was then used in solid phase synthesis to generate oligomers.

Example 3

Preparation of Oligomers Containing 7-deaza-2'-deoxyguanosine

In a manner similar to that set forth in Example 2, 2'-deoxy-7-deazaguanosine was converted to the 5'-DMT-blocked 3'-H-phosphonate monomer incorporated into oligomers.

Example 4

Binding of Oligomers of the Invention to Target Sequences

The double-stranded HER-2 target sequence used was:

5' AGGAGAAGGAGGAGG 3',

3' TCCTCTTCCTCCTCC 5'.

The GT oligomer sequence used was:

5' GGTGGTGGTTGTGGTY 3',

The GX oligomer sequence used was:

5' GGXGGXGGXXGXGGXY 3'.

Y is anthraquinone and was incorporated into oligomers as described (Lin, K. et al., Nucleic Acids Res (1991) 19:3111-3114). The anthraquinone moiety did not affect binding of the oligomer to duplex target DNA.

Both oligomers were footprinted, the GX oligomer footprinted at 1 μm; no binding was observed with the GT control oligomer. Triplex formation conditions were 20 mM MOPS, pH 7.2, 140 mM KCl, 1 mM spermine, and 1 mM $MgCl_2$. Thus, triplex formation was observed under physiological ion conditions due to the presence of 2'-deoxy-7-deazaxanthosine residues.

A G*T oligomer synthesized was

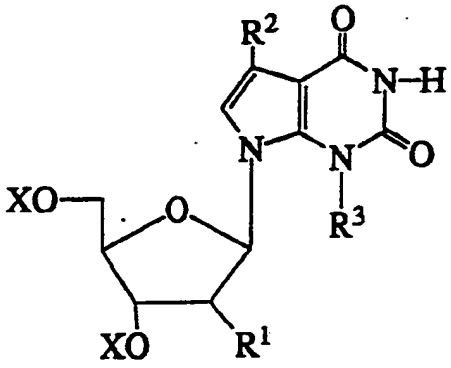
5'-G*G*TG*G*TG*G*TTG*TG*G*T-3'.

Under physiological ion and pH conditions, a triplex is formed with the target duplex.

The instant invention is shown and described herein in what is considered to be the most practical and preferred embodiments. It is recognized, however, that departures may be made therefrom which are within the scope of the invention, and that modifications will occur to those skilled in the art upon reading this disclosure.

CLAIMS

1. A compound of the formula:



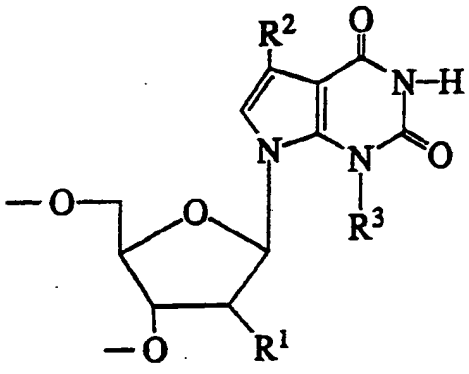
I

wherein R^1 is H, OH, F, Cl, O-allyl, S-allyl, OR or SR, wherein R is alkyl (1-4C); R^2 is H, alkyl(1-4C), CN, Br, Cl, F, $CONR_2$, lower alkenyl(1-4C) or lower alkynyl(1-4C); R^3 is H or a lower alkyl(1-4C); with the proviso that if R^2 and R^3 are both H, R^1 cannot be H or OH, and

wherein each X is independently H, $-PO_3^{-2}$ or a group useful in oligomer synthesis.

2. The compound of claim 1 wherein R^1 is H or OH, R^2 is CH_3, and R^3 is H.

3. An oligomer comprised of a plurality of nucleotide monomer units wherein at least one monomer unit comprises the moiety of formula:



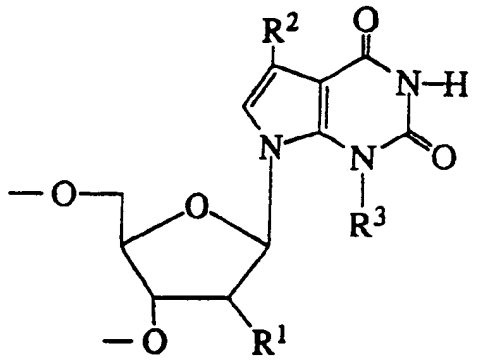
I(a)

wherein R^1 is H, OH, F, Cl, O-allyl, S-allyl, OR or SR, wherein R is a lower alkyl(1-4C); R^2 is H, alkyl(1-4C), CN, Br, Cl, F, $CONR_2$, lower alkenyl(1-4C) or lower alkynyl(1-4C); and R^3 is H or alkyl (1-4C).

4. The oligomer of claim 3, wherein the oligomer includes 2 to 30 monomer units and 50% or less of those monomer units comprise the moiety of formula I(a).

5. The oligomer of claim 3 wherein R^1 is H or OH, R^2 is CH_3, and R^3 is H.

6. A triplex of three oligomers wherein one of the three oligomers of the triplex is comprised of a moiety of the formula:



I(a)

wherein R^1 is H, OH, F, Cl, O-allyl, S-allyl, OR or SR, wherein R is lower alkyl(1-4C); R^2 is H, alkyl(1-4C), CN, Br, Cl, F, $CONR_2$, lower alkenyl(1-4C) or lower alkynyl(1-4C); and R^3 is H or lower alkyl(1-4C).

7. The triplex of claim 6, wherein R^1 is H or OH, R^2 is CH_3 and R^3 is H.

8. A method of treating a disease, which disease is characterized by a particular DNA duplex, the method comprising:

administering to a subject in need of such treatment a therapeutically effective amount of the oligomer of claim 3; and

allowing the oligomer to have sufficient time to bind to the DNA duplex.

9. A method of detecting the presence, absence or amount of a particular DNA duplex in a biological sample, comprising the steps of:

contacting the sample with the oligomer of claim 3 under conditions wherein a triplex is formed between the oligomer and the duplex DNA; and

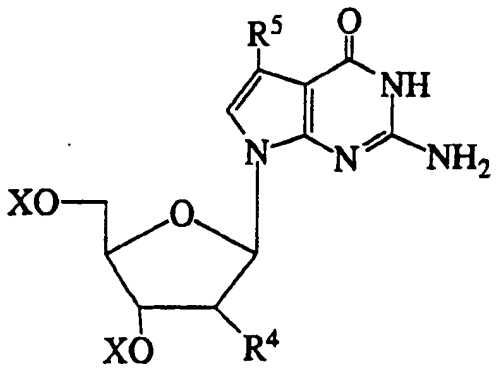
detecting the presence, absence or amount of said triplex.

10. A pharmaceutical composition, comprising:

a pharmaceutically acceptable carrier; and

a therapeutically effective amount of the oligomer of claim 3.

11. A compound of the formula:



II

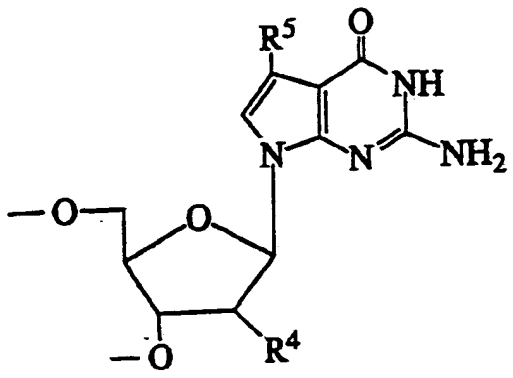
wherein R^4 is H, OH, F, Cl, O-allyl, S-allyl, OR or SR, wherein R is lower alkyl(1-4C); R^5 is H, lower alkyl(1-4C), CN, Br, Cl, F, $CONR_2$, lower alkenyl(1-4C) or lower alkynyl(1-4C); with the proviso that R^5 is not H when R^4 is H or OH;

wherein each X is independently H, $-PO_3^{-2}$ or a group useful in oligomer synthesis.

12. The compound of claim 11, wherein R^4 is H or OH and R^5 is lower alkyl.

13. The compound of claim 12 wherein R^5 is CH_3.

14. An oligomer comprised of a plurality of nucleotide monomer units wherein at least one said monomer unit comprises the moiety of formula II(a):



II(a)

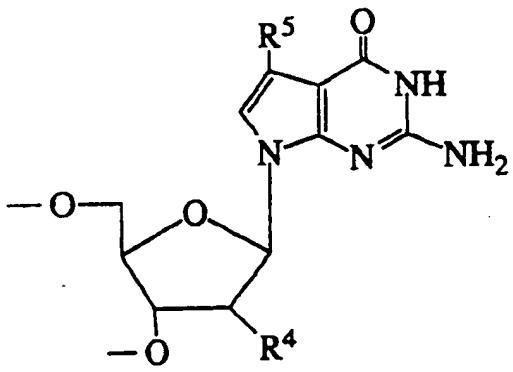
wherein R^4 is H, OH, F, Cl, O-allyl, S-allyl, OR or SR, wherein R is lower alkyl(1-4C); and R^5 is H, lower alkyl(1-4C), CN, Br, Cl, F, $CONR_2$, lower alkenyl(1-4C) or lower alkynyl(1-4C).

15. The oligomer of claim 14, wherein R^4 is H or OH and R^5 is lower alkyl.

16. The oligomer of claim 15 wherein R^5 is CH_3.

17. The oligomer of claim 14, wherein the oligomer includes 2 to 30 monomer units and 50% or less of those monomer units comprise the moiety of formula II(a).

18. A triplex of three oligomers wherein one of the three oligomers of the triplex is comprised of a moiety of formula II(a):



II(a)

wherein R^4 is H, OH, F, Cl, O-allyl, S-allyl, OR or SR, wherein R is lower alkyl(1-4C); and R^5 is H or lower alkyl(1-4C) CN, Br, Cl, F, $CONR_2$, lower alkenyl(1-4C) or lower alkynyl(1-4C).

19. The triplex of claim 17 wherein R^5 is alkyl and R^4 is H.

20. The triplex of claim 17, wherein R^5 is CH_3.

21. A method of treating a disease, which disease is characterized by a particular DNA duplex, the method comprising:

administering to a subject in need of such treatment a therapeutically effective amount of the oligomer of claim 14; and

allowing the oligomer to have sufficient time to bind to the DNA duplex.

22. A method of detecting the presence, absence or amount of a particular DNA duplex in a biological sample, comprising the steps of:

contacting the sample with the oligomer of claim 14 under conditions wherein a triplex is formed between the oligomer and the duplex DNA; and

detecting the presence, absence or amount of said triplex.

23. A pharmaceutical composition, comprising:
a pharmaceutically acceptable carrier; and
a therapeutically effective amount of the oligomer of claim 14.

24. The compound of claim 1 wherein at least one X is a group useful in oligomer synthesis.

25. The compound of claim 24 wherein the group useful in oligomer synthesis is selected from the group consisting of DMT, MMT, H-phosphonate, methyl phosphonate, β-cyanoethylphosphoramidite and methylphosphoramidite.

26. The compound of claim 11, wherein at least one X is a group useful in oligomer synthesis.

27. The compound of claim 26, wherein the group useful in oligomer synthesis is selected from the group consisting of MMT, DMT, H-phosphonate, methyl phosphonate, β-cyanoethylphosphoramidite or methylphosphoramidite.

28. The oligomer of claim 3 comprising at least one region of inverted polarity.

29. The oligomer of claim 28, comprising at least one o-xyloso linker.

30. The oligomer of claim 14 comprising at least one region of inverted polarity.

31. The oligomer of claim 30 comprising at least one o-xyloso linker.

32. The oligomer of claim 3 comprising at least one GT motif binding region and at least one CT motif binding region.

33. The oligomer of claim 14 comprising at least one GT motif binding region and at least one CT motif binding region.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/09195

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C07H 21/00, 19/00; C12Q 1/68; A61K 48/00
US CL :536/27; 435/6; 514/44
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/27; 435/6; 514/44

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS ONLINE, MEDLINE, APS, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y,P A	Proceedings of the National Academy of Sciences, Volume 89, issued 01 May 1992, S. T. Krawczyk et al., "Oligonucleotide-mediated triple helix formation using an N3-protonated deoxycytidine analog exhibiting pH-independent binding within the physiological range", pages 37-61-3763, see entire document.	1-7,11-20,24-33 8-10,21-23
X	Liebigs Annals of Chemistry, Volume 4, issued 1984, H. D. Winkeler et al., "Synthesis and furanoside/pyranoside isomerization of 7-deaza-2'-deoxy-7-methylguanosine", abstract.	11-13,26
Y	EP,A, 0,375,408 (Hogan et al.) 27 June 1990, especially pages 5,8 and examples 1-14.	1-33
Y	Journal of the American Chemical Society, Volume 112, issued 1990, D. A. Horne et al., "Recognition of Mixed-Sequence Duplex DNA by Alternate-Strand Triple-Helix Formation", pages 2435-2437, see entire document.	28-33

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be part of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 NOVEMBER 1992	2 DEC 1992
Name and mailing address of the ISA/ Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer LISA T. BENNETT Telephone No. (703) 308-0196

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/09195

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Proceedings of the National Academy of Sciences, Volume 85, issued 01 March 1988, D. Praseuth et al., "Sequence-specific binding and photocrosslinking of alpha and beta oligonucleotides to the major groove of DNA via triple-helix formation", pages 1349-1353, see entire document.	1-33
A	Science, Volume 241, issued 22 July 1988, M. Cooney et al., "Site-specific oligonucleotide binding represses transcription of the human c-myc gene in vitro", pages 456-459, see entire document.	1-33
A	Science, Volume 251, issued 15 March 1991, P. A. Beal et al., "Second structural Motif for recognition of DNA by oligonucleotide-directed triple helix formation", pages 1360-1363, see entire document.	1-33